Original article

# The G1057D Polymorphism of Insulin Receptor Substrate-2 *(IRS2)* Gene With Type 2 Diabetes in the Turkish population

**(Türk populasyonununda tip 2 diabet hastalarında insülin substrat-2 geni g1057d polimorfizmi)**

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**ÖZET**

**Amaç:** İnsülin reseptör substratı (IRS) proteini, hedef dokulara insülin sinyalinin iletilmesinde kritik bir öneme sahiptir. IRS-2 knockout farelerin insanlardaki tip 2 diabete benzer bir klinik fenotip gösterdiği tespit edilmiştir. IRS2 geninde çok sayıda polimorfizm belirlenmiş olmakla birlikte farklı populasyonlarda tip 2 diabet ile IRS-2 Gly1057Asp polimorfizmi arasındaki ilişki araştırılmış olup elde edilen sonuçlar oldukça çelişkilidir. Bu çalışmada Türk populasyonunda Diabetes mellitus ile IRS-2 G1057D polimorfizmi arasındaki ilişkiyi araştırmak amaçlanmıştır.

**Materyal ve Metot:** Çalışma populasyonu 328 kişiden oluşmakta olup 138 kişi DM hastası ve 190 kişide kontroldür. DNA periferik kandan izole edilmiş olup IRS2 G1057D polimorfizmi PCR-RFLP yöntemi kullanılarak belirlenmiştir.

**Bulgular:** IRS2 G1057D polimorfizmi genotip dağılımlarının gruplar arasında istatistiksel olarak anlamlı bir farklılık göstermediği tespit edilmiştir.

**Sonuçlar:** Elde edilen bulgular IRS2 G1057D polimorfizminin tip 2 diabetin patogenezinde rolü olmadığını göstermektedir.

**Anahtar Kelimeler:** İnsülin substrat 2; polimorfizm; tip II diabet

**ABSTRACT**

**Background:** Insulin receptor substrate (IRS) proteins are critical to signal transduction in insulin target tissues. IRS-2 knockout mice exhibit a phenotype similar to human type 2 diabetes, characterized by insulin resistance with abnormal glucose tolerance at birth culminating in the development of fasting hyperglycemia in later age. While several polymorphisms have been identified in the IRS-2 gene, the association of the Gly1057Asp polymorphism with type 2 diabetes has been studied in different populations, but the results have been inconsistent. The present study was undertaken to determine IRS-2 G1057D polymorphism association with Diabetes mellitus in Turkish subjects.

**Material and Methods:** The study population consisted of 328 subjects 138 patients with DM and 190 controls. DNA was extracted from peripheral blood. Genetic polymorphism of IRS-2 G1057D was detected by using PCR-based restriction fragment-length polymorphism.

**Results:** The genotype distribution of the IRS2 G1057D polymorphism was not statistically significant between groups.

**Conclusions:** These results strongly argue against a major role of the G1057D IRS-2 polymorphism in the pathogenesis of type 2 diabetes in Turkish subjects.

**Key Words:** Insulin receptor substrate-2; polymorphism; diabetes mellitus

## INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous disorder characterized by the presence of chronic hyperglycemia. Maintenance of normal glucose homeostasis involves the action of a glucose sensor in the pancreatic beta cell that detects an increase in blood glucose concentration and converts that into increased secretion of insulin[1,2].

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The insulin signalling pathway begins with the binding of insulin to the $\alpha$-subunit of the insulin receptor and ends with the biological effects of insulin in multiple tissues[3].

The two key pathophysiological defects of type 2 diabetes are impaired insulin signalling and failure of beta cells to compensate for the increased insulin demand. Longitudinal studies suggest that insulin resistance is the earliest detectable defect in the pathogenesis of type 2 diabetes[4,5]. Accordingly, genetic variants of molecules involved in insulin signalling and beta cell function may play a role in the pathogenesis of the disease. In particular, the insulin receptor substrate-2 (*IRS-2*), one of the major substrates

of the insulin receptor, may be an attractive candidate in the pathogenesis of type 2 diabetes, given its crucial role in insulin signalling[6] and in beta cell development and/or survival[7]. Together with *IRS-1*, it mediates most insulin effects, especially those associated with somatic growth and carbohydrate metabolism. Disruption of *IRS-2* was shown to cause type-2 diabetes in mice[8]. Thus, *IRS-2*-null mice exhibit severely impaired glucose metabolism, exemplified by peripheral insulin resistance and impaired proliferation and/or enhanced apoptosis of pancreatic β cells[7] and[9], a phenotype resembling that of diabetes in humans.

In humans, a number of polymorphisms have been identified in the *IRS-2* gene. Only three variants (Leu647Val, Gly897Ser, and Gly1057Asp substitutions) have been previously reported in the IRS-*2* gene in Caucasian subjects[10,11]. Studies show that homozygous carriers of the Gly1057 allele had higher 2-h plasma glucose concentrations during an oral glucose tolerance test (OGTT)[12]. Also in different ethnic groups, *IRS-1* polymorphic variants prevalence was found to be slightly higher in type 2 diabetes than in nondiabetic control subjects[13-18]. To our knowledge, this is the first report on this subject for the Turkish population. The aim of this study was to investigate the association of Gly1057Asp (G1057D) polymorphism in *IRS-2* gene among Turkish patients with diabetes.

## MATERIAL and METHODS

One hundred thirty eight patients with DM (60 males and 78 females) and 190 control subjects (92 males and 98 females) were studied. The two groups were matched with respect to age and body mass index (BMI) determined from the weight and height of the patients. Patients were chosen from the outpatients clinic of the Endocrinology Department of the Diabetes Center in Mersin. Diagnosis of diabetes was made according to revised American Diabetes Association criteria[19]. None of the patients had a history of cerebrovascular or ischemic heart diseases, neuropathy, renal dysfunction and hypertension. Control subjects were selected from among healthy people with no history of cardiovascular disease, cancer, diabetes and hypertension. All the subjects gave written informed consent. The research protocol was approved by the Ethics Committee of Mersin University.

*IRS-2 genotyping*

Genomic DNA was extracted from 200 μl of peripheral blood by High Pure DNA isolation Kit (Qiagen, Inc., Chatsworth, CA) following manufacturer instructions. A polymerase chain reaction (PCR)-based restriction fragment-length polymorphism (RFLP) method was used to genotype *IRS-2* G1057D polymorphism, which removes Hae II restriction enzyme site. The PCR was performed in a 25 μl volume containing 20 ng genomic DNA, 10XPCR buffer with 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 10% Dimethylsulphoxide, 0.5 units of Taq polymerase (Fermantas, MBI), and 5 pmol of each primers *IRS-2*F (5' GCT CCC CCA AGT CTC CTA A 3') and *IRS-2*R (5' CTC AGC CTC TTC ACG CCC 3'). The PCR thermal cycling conditions were an initial melting period at 95°C for 2 min; then 35 amplification cycles of 95°C for 45 s, 62°C for 45 s and 72°C for 45 s; and a 7-min extension step at 72°C. The PCR products were checked on a 1.5% agarose gel for the assay completion and then the PCR products of 375 base pair (bp) were digested with restriction enzyme Hae-II by overnight incubation at 37°C. The digestion products were electrophoresed on 3% agarose gel and visualized by staining with ethidium bromide and evaluated using the gel documentation system (Vilber-Lourmat, Cedex, France).

A case-control study was performed and allelic frequency of the polymorphism was calculated both in cases and controls. The $\chi^2$ test was used to compare genotype frequency of the *IRS-2* gene polymorphism between DM patients and controls. The association between *IRS-2* polymorphisms and DM was modeled through binary logistic regression analysis and odds ratio (OR) and 95% confidence interval (95%CI) were calculated to compare DM risk around genotypes. *P* value less than 0.05 was considered as significantly different. The software used for the calculation was the SPSS version 11.5 (SPSS Inc., Chicago, IL).

## RESULTS

A total of 138 patients with DM (mean age, 56.36±13.03 years) and 190 controls subjects (mean age, 57.12±14.56 years) participated in this study. Clinical characteristics of both populations are shown in Table 1.

**Table 1.** The Distribution of the Patients and the Controls by age and Gender

| | Patients | Controls | P value |
|---|---|---|---|
| **Total number** | 138 | 190 | |
| Age (mean of years±SD) | 56.36±13.03 | 57.12±14.56 | p=0.60 |
| **Gender** | | | p=0.43 |
| Female n (%) | 78 (56.5) | 98 (51.6) | |
| Male n (%) | 60 (43.5) | 92 (48.4) | |

n: Number of sample

**Table 2.** *IRS-2* Genotype and Allele Frequencies of the Patients and the Controls

| | **Patients (n=138)** | **Controls (n=190)** | | |
|---|---|---|---|---|
| | n (%) | n (%) | OR ǂ | 95% CI |
| **Genotype** | | | | |
| GG | 61 (44.2) | 85 (44.7) | 1 (Reference)* | |
| GD | 57 (41.3) | 84 (44.2) | 0.94 | 0.59-1.51 |
| DD | 20 (14.5) | 21 (11.1) | 1.32 | 0.66-2.65 |
| **Allele** | | | | |
| G | 179 (64.9) | 254 (66.8) | 1 (Reference)* | |
| D | 97 (35.1) | 126 (33.2) | 1.09 | 0.78-1.51 |

n: Number of sample
OR ǂ (odds ratio); 95% CI (95% confidence interval) from conditional logistic regression
* Carriers of at least one intact allele are used as reference

As shown in Table 2; for *IRS-2* G1057D polymorphism, the frequencies of GG, GD and DD were 44.2, 41.3 and 14.5% in cases and 44.7, 44.2 and 11.1% in controls. We did not find any difference between the genotypes in groups ($p>0.05$; Table 2).

The frequency of G allele was 0.649 for cases and 0.668 for controls. As for the frequency of D allele was 0.351 for cases and 0.332 for controls. There was no difference between cases and controls in the frequency of G and D alleles ($p>0.05$; Table 2).

## DISCUSSION

DM is the most common metabolic disorder and results from the interaction between genetic and environmental factors. *IRS-2* plays an important role in insulin signalling and its disruption, in mice, results in diabetes[7,20]. This could be attributed largely to hepatic insulin resistance and lack of beta cell compensation. IRS-2 knockout mice (IRS-$2^{-/-}$) exhibit nearly normal birth size and body weight, but show insulin resistance with abnormal glucose tolerance at birth and progressively develop fasting hyperglycemia as a result of inadequate compensatory insulin secretion because of reduced ß cell mass[6,7]. So it can be hypothesized that a lack of *IRS-2* is especially important in the development of DM and we investigated the significance of the variants of the *IRS-2* gene in patients with type 2 diabetes. But we did not observe an association between the *IRS-2* genotypes and an increased risk for DM. This supports the findings of many publications[10,21-27]

However, in a study with Italian population reported an association of this polymorphism with lower risk of diabetes in Italian people, but with a higher risk in obese people suggesting that there could be a gene-environment interaction. In that study, the D1057 allele was negatively associated with type-2 diabetes among patients who were not obese, but positively associated with the disease among those who were, implying the D1057 allele increases the risk of diabetes among obese individuals. A different genetic background as clearly suggested by the difference in allelic frequency may also play a role[28].

A study in Pima Indians also suggested that the presence of obesity could influence the susceptibility of this polymorphism to diabetes.

Also in Pima Indians, the frequency of the Asp1057 allele of the Gly1057Asp polymorphism in *IRS-2* is higher than in any other population reported to date[29].

It may be that, in the diabetic state, the D1057 allele have a detrimental effect on insulin sensitivity in obese patients by impairing *IRS-2*-mediated signaling in hypothalamus, which would in turn exacerbate their leptin resistance. By contrast, the allele may have no effect on insulin sensitivity in patients who are not obese[25,30,31].

Also there is no information on the effect of this polymorphism on the molecular function of *IRS-2*, it is hypothesised that this introduces a charged amino acid (D) in place of a neutral one (G) in the domain of IRS-2 molecule located in between two putative tyrosine phosphorylation sites (at positions 1042 and 1072) of the protein which could produce alterations in downstream signalling through IRS-2[10,11]. However, there is the possibility that this polymorphism is not functional but may be in linkage disequilibrium with a currently unrecognized functional polymorphism.

In conclusion our results strongly argue against a major role of the G1057D *IRS-2* polymorphism in the pathogenesis of type 2 diabetes in Turkish Population.

(**Conflict of interest of statement:** None declared)

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